Patent Docket P1101P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	Group Art Unit: 1647
Avi J. Ashkenazi	Examiner: C. Kaufman
Serial No.: 09/020,746	
Filed: February 9, 1998	
For: Apo-2 Receptor Antibody	

RULE 131 DECLARATION

I, Avi J. Ashkenazi, hereby declare as follows:

1. I am the named inventor of the claimed subject matter of the above-identified patent application.

2. The above-identified patent application claims priority to application serial no. 08/857,216 filed with the Patent Office on May 15, 1997, and I am the named inventor in that priority application. A copy of my priority application serial no. 08/857,216 is attached as Exhibit A.

3. All work described in the above-identified application, in the priority application (Exhibit A), and as described below and in the laboratory notebook pages attached as Exhibits B and C was performed by me or on my behalf in the United States of America.

4. As stated in my previous Declarations filed in the above-identified application, I have reviewed, among others, the following patent application documents filed in the name of SmithKline Beecham Corporation, Human Genome Sciences and Millennium Pharmaceuticals, respectively: application no. 60/041,230 filed March 14, 1997; application no. 60/040,846 filed March 17, 1997; and application no. 08/843,652 filed April 16, 1997.

5. Prior to March 14, 1997, experiments were conducted by me or under my direction which resulted in the identification and

characterization of a receptor molecule referred to in my priority application and the present application as "Apo-2". Among other things, these experiments resulted in the determination of the full-length cDNA and putative amino acid sequences of Apo-2, and that overexpression of Apo-2 in mammalian cells induced apoptosis. See, for example, copies of laboratory notebook pages from Notebook Nos. 26865, issued to Scot Marsters and 26508, issued to James Sheridan, which are attached hereto as Exhibits B and C. All dates identified on the attached pages in Exhibits B and C are prior to March 14, 1997 and have been redacted.

6. Prior to March 14, 1997, a clone having certain homology to TNF receptor family members Apo-3 and Fas was identified using database searching techniques. In an *in vitro* experiment performed by my Senior Research Associate, Scot Marsters, this clone (referred to as "DD2.1") was transfected into 293 cells and tested for its ability to induce cell death. As shown on page 47 of Notebook No. 26865 (Exhibit B), overexpression of DD2.1 (in Plate # 15) was found to kill the transfected cells (i.e., "2.1 kills cells").

7. Also prior to March 14, 1997, my Post-doctoral research fellow, James Sheridan, conducted an *in vitro* experiment in which the clone DD2.1 (referred to above) was transfected into HeLa cancer cells together with a plasmid encoding CD4 as a transfection marker and the cells were stained with PE-conjugated CD4 antibody to identify transfected cells and with FITC-conjugated Annexin V to identify apoptotic cells and analyzed by fluoroscense-activated cell sorting (FACS). (Sheridan Notebook No. 26508, at page 83, Exhibit C). In the experiment, samples containing CRMA or FADD-DN were also analyzed. CRMA is a viral inhibitor of caspases used to block apoptosis by inhibiting caspase activation by death receptors. FADD-DN is a dominant negative mutant of the FADD adaptor protein that was known at the time to block apoptosis induction by TNF receptors TNFR1, Fas and Apo-3. On page 83, clone DD2.1 is referred to as "2-1" (see sample #3) and the finding (discussed in paragraph 6 above) that the clone killed 293 cells is noted (i.e. killed 293s). Results of the HeLa experiment are noted on the right hand side of page 83 as % PE positive % FITC positive cells (i.e. transfected and apoptotic), the control (sample #1) being 14.1%; clone 2-1 (sample #3) being 35.4%; clone 2-1 plus CRMA (sample #11) being 11.4%; and clone 2-1 plus FADD-DN (sample #15) being 28.9%. Near the bottom

of page 83, James Sheridan indicates that overexpression of 2-1 caused apoptosis, that this could be blocked by CRMA, and that this could be reduced by Hu FADD-DN.

8. The full-length cDNA and putative amino acid sequences of clone DD2.1 were identified prior to March 14, 1997. Those putative amino acid and encoding cDNA sequences are the same sequences disclosed in my priority patent application (Exhibit A) (see, e.g., Figure 1 of Exhibit A).

9. The nomenclature of the molecule referred to above as DD2.1 (or 2.1) was changed to "Apo-2", and the molecule is referred to in both my priority application (Exhibit A) and the above-identified patent application as Apo-2.

10. Prior to March 14, 1997, I believed the Apo-2 molecule to be a novel member of the TNF receptor family. Prior to March 14, 1997, I also recognized that the Apo-2 molecule contained a death domain and was capable of inducing apoptosis in cells.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/25/03
Date

Avi Ashkenazi
Avi J. Ashkenazi, Ph.D.